Anaerobic Bacteria and Biogas Volume in POME and PPF Mixed Media with Starter from Buffalo Feces

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Abstract. The buffalo population until 2018 was recorded at 1,356,390 heads throughout Indonesia. If buffalo feces are not appropriately managed, it will cause problems in the form of environmental pollution. Buffalo feces has the potential because it contains microorganisms that can be used to produce biogas. Starter from buffalo feces can also add to organic media from plantation waste. The process of oil extraction, washing, and cleaning at the factory produces palm oil mill effluent (POME). POME contains low carbon, so for biogas formation in an anaerobic digester, it is necessary to add a carbon source. The carbon source can obtain from palm-pressed fiber (PPF), also a solid waste of the palm oil industry. Adding a starter from buffalo feces in a mixture of POME and PPF media is expected to reduce waste that has yet to be utilized optimally by buffalo farms and the oil palm industry. This study was conducted to determine the effect of adding the best starter from buffalo feces to a mixture of POME and PPF media on the number of anaerobic bacteria and biogas volume. The study used a completely randomized experimental design (CRD) with five treatments, namely P1 (without the addition of starter), P2 (+ 2% starter), P3 (+ 4% starter), P4 (+ 6% starter), and P5 (+ 8% starter) for 28 days with observations on days 0, 7, 14, 21, 28. Each treatment was repeated four times. The observed variables were the number of anaerobic bacteria and the volume of biogas.

Keywords: biogas · buffalo · POME · PPF · starter

1 Introduction

The buffalo population until 2018 recorded at 1,356,390 heads throughout Indonesia. The large amount of buffalo feces produced is largely untapped. If buffalo feces are not managed properly, it will cause problems in the form of environmental pollution. Buffalo feces has potential because it contains microorganisms that can be used as a starter in an anaerobic digester to produce biogas. Starters from buffalo feces can also add to organic media from plantation waste. One example is palm oil industry waste. The increase in domestic palm oil production is not matched by optimal waste management. The process of oil extraction, washing, and cleaning at the factory produces palm oil mill effluent (POME).

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Almost all palm oil mills in Indonesia use an open pond system to process POME. Although pond systems are economically advantageous, they facilitate the release of methane into the atmosphere. This occurs due to the anaerobic decomposition process because sunlight does not penetrate the top layer of POME, which is accommodated in an open pond. Therefore, POME has the potential to be used as a medium for biogas formation. POME analyzed contains low carbon, so for biogas formation in an anaerobic digester, it is necessary to add a carbon source. The carbon source can be obtained from palm pressed fiber (PPF), also a solid waste of the palm oil industry.

Adding a starter from buffalo feces in a mixture of POME and PPF media is expected to reduce waste that has not been utilized optimally by buffalo farms and the oil palm industry. Based on the description above, it is necessary to research the number of anaerobic bacteria and the volume of biogas in a mixture of POME and PPF media that was given a starter from buffalo feces.

2 Materials and Methods

The materials used in this study were buffalo feces, POME, PPF, beef cattle rumen fluid, and Media. The study consisted of three stages, namely (I) preliminary in vitro procedure and adaptation aimed at obtaining a starter to be used for treatment, (II) using a starter according to the dose of each treatment into a serum bottle containing a mixture of POME and PPF media. (C/N = 27.37%), (III) performed randomization to obtain results that guarantee the validity of the unbiased assumption of experimental error, the mean treatment value, and the difference between them.

The design used in this study was a completely randomized design (CRD) with a dose consisting of five treatments, and each treatment was repeated four times so that the total experimental units were 20 units. The composition of the treatment that will be tested is as follows:

P1 = 125 ml POME + 20% PPF.
P2 = 125 ml POME + 20% PPF + 2% starter from buffalo feces.
P3 = 125 ml POME + 20% PPF + 4% starter from buffalo feces.
P4 = 125 ml POME + 20% PPF + 6% Starter from buffalo feces.
P5 = 125 ml POME + 20% PPF + 8% starter from buffalo feces.

The observed variables were the number of anaerobic bacteria and the volume of gas produced.

3 Results and Discussion

3.1 Effect of Treatment on the Number of Anaerobic Bacteria

The average number of anaerobic bacteria in POME and PPF media without the addition of starter 2, 4, 6, and 8% from buffalo dung ranged from 2.41–3.03 x 10^{12} CFU/ml. The highest average number of anaerobic bacteria was found in P5 (8% starter from buffalo dung), and the lowest was in P1 (without the addition of starter). This result is affected because POME contains microorganisms, and PPF is challenging to hydrolyze. Therefore, POME can serve as a source of nutrition for microorganisms. In the opinion of Choong et al. [4] POME contains microorganisms that cause the added starter to require another adaptation process. This then triggers competition for resources (substrate nutrients) between microorganisms from POME and the starter. POME is an easily hydrolyzed substrate compared to PPF. Research on bacterial isolation of palm oil industrial wastewater (POME) has been carried out by Januar et al. [11], they reported that isolation results from POME obtained nine isolates of lipid-degrading bacteria, with one isolate able to reduce lipid levels by 25%. Bala et al. [1] also obtained a strain of Bacillus cereus 103PB which has biodegradability and can reduce pollutants from palm oil industrial waste. These bacteria produce extracellular lipase enzymes to reduce waste oil and fat levels.

Microorganisms will utilize PPF as a carbon source in the hydrolysis process for energy sources. Analysis of the Laboratory of Soil Chemistry and Plant Nutrients (KTNT) Faculty of Agriculture Unpad (2019) found an organic C content in PPF of 59.44%. The cellulose and lignin contents in PPF are 32.4 and 20.5%, respectively. PPF, on the other hand, is a carbon source that is difficult to use because it has not received any treatment that can help break down the crude fiber in PPF. This can be seen from the 14th to the 21st day. There was a decrease in the number of anaerobic bacteria in the media due to the relatively slow decomposition of carbon sources. Deublein and Steinhauser [6] state that the hydrolysis stage is the first step in forming biogas and decomposing complex organic matter into simpler compounds. The presence of lignin in mixed media can inhibit the utilization of hydrolyzed nutrients by bacteria that are useful for breeding.

Lignin is one of the inhibitors of the hydrolysis process carried out by anaerobic bacteria. Therefore, the complex structure of lignin needs to be processed first. This is based on the results of research by Iranmahboob et al. [10] that natural cellulose is bound by hemicellulose and protected by lignin. The presence of lignin-binding compounds makes this material challenging to hydrolyze, considering not only physical pre-treatment but also chemical pre-treatment with the addition of NaOH to destroy the lignocellulosic components in PPF. The aim is to open up the lignocellulosic structure so that the cellulose becomes more accessible to enzymes that break down the saccharide polymers into sugar monomers. Pre-treatment provides easier access to enzymes, increasing glucose and xylose yields Mosier et al. [14].

Figure 1 shows the occurrence of bacterial reproduction, which is characterized by an increase in the quantity of cell mass through the formation of new cells or individual growth of the number of anaerobic bacteria, which is influenced by physical or chemical factors from the environment. This statement is in line with the opinion of Tortora et al. [19] that the main factors affecting bacterial growth are nutritional factors (available substrate) and physical factors (moisture content, temperature, and pH).

During biogas formation, many bacteria are involved in various stages, having different requirements in terms of habitat, as the microorganisms responsible for hydrolysis and acidogenesis are facultative and obligate anaerobic bacteria [12, 18, 20]. This condition is different from methanogenic microorganisms, the weakest chain in biocenosis, because their growth rate is low and they are the most sensitive to disturbance. Therefore,

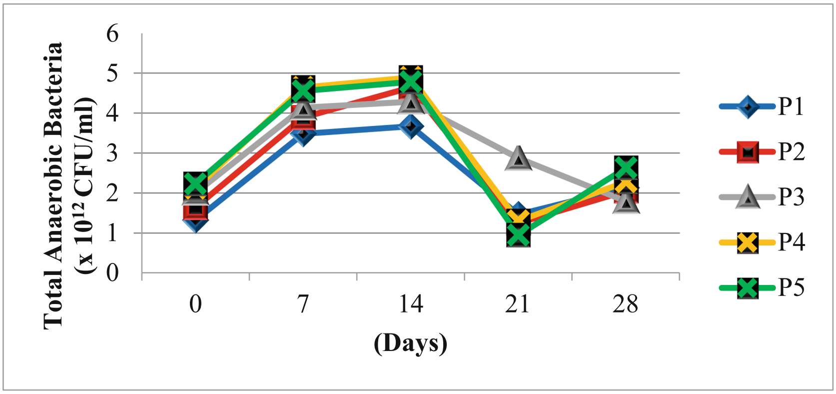

Fig. 1. Graph of Total Anaerobic Bacteria

the environment must be adapted to the needs of methane-forming bacteria [16]. In addition, according to Miyamoto [13] and Tortora et al. [19], all methanogens are archaea and obligate anaerobes, which require a redox potential below 300 mV for growth. They are sensitive to oxygen and grow very well between H2 and CO2. Therefore, these microorganisms still depend on an environment without oxygen. Most species are killed by small amounts of oxygen [16].

3.2 Effect of Treatment on Biogas Volume

The average volume of biogas in POME and PPF media without adding 2, 4, 6, and 8% starter from buffalo dung ranged from 20.95–52.00 ml. The highest average volume of biogas was found in experiment P5, and the lowest was in the control or P1. The formation of gas indicates the occurrence of microbial metabolism.

Based on the illustration in Fig. 2. it can be seen that from day 0 to day 7, gas production increased dramatically, with day 7 being the peak volume of biogas production. However, after the seventh day, gas production decreased significantly in all treatments up to the 21st and slightly increased on the 28th.

The increase in biogas volume, along with the addition of starter content, was caused by the increase in the mass of microorganisms due to the adaptation of the starter from buffalo dung which had previously been carried out. In addition, the starter contains microorganisms ready to play a role in the anaerobic decomposition process and work more efficiently [9]. However, the adaptation time of the starter on the substrate (POME and PPF) may be disturbed because, according to Choong et al. [4], POME contains microorganisms that can create competition with starters when utilizing existing nutrient sources. This result is also affected because PPF contains lignocellulosic compounds, making it difficult to hydrolyze.

During the biogas formation process, it was reported that there were microorganisms that played a role in hydrolysis and acidogenesis, namely several bacterial genera, including Clostridium, Peptococcus, Bifidobacterium, Desulphovibrio, Corynebacterium, Lactobacillus, Actinomyces, Staphylococcus and Escherichia coli from anaerobic digesters [12, 18, 20].

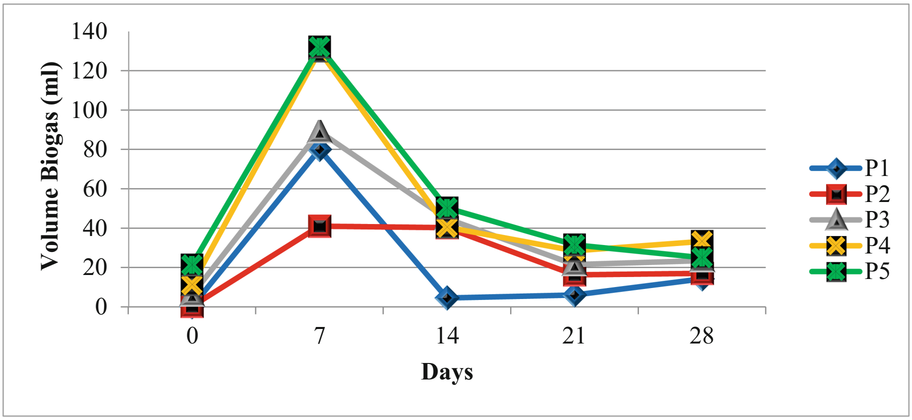

Fig. 2. Biogas Volume Chart

Microorganisms contained in POME and starter require a source of nutrition in mixed media. Therefore, both of them can carry out anaerobic metabolism (fermentation), which can degrade polysaccharides, proteins, and lipids originating from the media. In this case, the organic acids produced through hydrolysis are then converted into CH4 gas by rumen archaeal methanogens [3]. Methanogenic archaea can produce CH4 from low-carbon substrates such as formate, pyruvate, methyl amine, acetate, and CO2 through methanogenesis.

The addition of a starter from buffalo manure did not affect the volume of biogas, possibly because the C/N ratio was too high. Nitrogen is consumed rapidly (in POME because it is readily hydrolyzed compared to PPF) by archaeal methanogens to meet their protein requirements. It is no longer available to react to the residual carbon content in materials. As a result, biogas production is under pressure [15] depicted in Illustration 2. After the seventh day, which tends to decrease.

4 Conclusions

Based on the results of the analysis and discussion, the following conclusions can be drawn: the average number of anaerobic bacteria given as starters from buffalo feces at a dose of 2%, 4%, 6%, and 8%, respectively, was 2.41; 2.69; 3.01; 3.05, and 3.03 x 1012 CFU/ml. Meanwhile, the average value of biogas volume is 20.95, 22.95; 36.95; 48.70, and 52.00 ml. The addition of starter from buffalo feces at doses of 2%, 4%, 6%, and 8% in a mixture of POME and PPF media did not increase the number of anaerobic bacteria and the volume of biogas.

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